A Systems Biology Approach to Personalizing Therapeutic Combinations

Lawrence N. Kwong1, Timothy P. Heffernan1,2, and Lynda Chin1,2

Summary: The identification of evidence-based, efficacious drug combinations for each cancer, among thousands of potential permutations, is a daunting task. In this perspective, we propose a systematic approach to defining such combinations by molecularly benchmarking a drug against a desired state of efficacy using model systems.

Cancer Discov; 3(12): 1339–44. © 2013 AACR.

INTRODUCTION

Although targeted therapies have revolutionized cancer treatment, the lack of durable responses has highlighted the need for combination regimens to overcome primary or acquired drug resistance. To date, the rational design of effective drug combinations has relied on knowledge-based assessments, high-throughput screens, or identification of presumed compensatory pathways after single-drug administration. More recently, we have reported on a data-driven strategy based on the concept of benchmarking against a desired phenotype. In an inducible NRAS mouse model of melanoma, the genetic extinction of oncogenic NRAS results in complete tumor regression, hence defining a desired “ideal” state. Benchmarking against such a molecular state allowed for identification of a drug combination that more closely simulates the efficacy of genetic NRAS extinction. In this perspective, we discuss the potential of generalizing this methodology to enable data-driven cotargeting therapeutic strategies against undruggable cancer targets, both oncogenes and tumor suppressors. Furthermore, we speculate on how this approach can be applied clinically to address the challenge of heterogeneity in patient responses, namely, a systematic approach to the designing of a combination strategy customized to a patient, by benchmarking the patient’s unique response to a drug against a predefined molecular state representing the desired efficacy. Such an adaptive approach to personalizing therapeutic combinations has the potential to delineate the clinical paths for durable complete responses in the clinic.

A BENCHMARK-DRIVEN APPROACH FOR THE DISCOVERY OF COTARGETING STRATEGIES

Systems biology—the data-driven network modeling of complex biologic systems—holds promise to identify novel cancer therapies in an unbiased manner. One recent study applied such modeling to predict that the apoptotic response in breast cancer is optimized by the sequential rather than simultaneous application of chemotherapy and an EGF receptor (EGFR) inhibitor (1). In another study, computational modeling identified ErbB3 as the most effective therapeutic target across the ErbB–PI3K axis, leading to the development of a novel and effective therapeutic ErbB3 antibody (2). Similarly, modeling of EGFR phospho-signaling identified MET plus EGFR inhibition as synergistic (3).

We have recently published (4) another example of a data-driven approach to the development of evidence-based therapeutic combinations (Fig. 1A). This study leveraged a mouse model of melanoma, engineered so that the expression of mutant NRAS can be extinguished via withdrawal of doxycycline; loss of mutant NRAS expression resulted in a rapid and complete tumor regression—the desired state. In contrast, pharmacologic inhibition of the RAS downstream effector MAP–ERK kinase (MEK), given at maximum-tolerated doses, was unable to phenocopy this response, failing to induce tumor regression and achieving only transient growth arrest. Global transcriptional and targeted proteomic profiling, validated by tumor histopathologic analyses, illuminated the differential molecular effects of genetic NRAS extinction versus pharmacologic MEK inhibition. Not surprisingly, mutant NRAS proved to be a tumor-maintenance target, as its activities were required for both growth and survival of an established tumor and its genetic extinction resulted in complete tumor regression. However, a potent and specific MEK inhibitor (hereafter MEKi) was only able to block the survival signal by mutant NRAS, consequently activating apoptosis, but failing to inhibit the proliferation signal. Therefore, drug(s) that can inhibit proliferation represented a possible rational combination with MEKi against mutant NRAS. To that end, network modeling was applied to discover key regulators underpinning these molecular differences in an unbiased manner. Here, we used TRAP (Transcriptional...
As a prime example of an undruggable target, the exact significance of NRAS (in vivo) and pharmacologic inhibition of MEK in the iNRAS system, this data-driven approach identified two drugs (MEK and CDK4 inhibitors) that synergize in combination to approximate the therapeutic efficacy of NRAS extinction. This general paradigm of benchmarking against a desired state to delineate potential synergistic combinations can be applied broadly (Fig. 1C). Indeed, various genetically engineered mouse models (GEMM) of oncogene addiction have already been established (6). The proof-of-concept example described above provides a potential path to define novel therapeutic strategies to target other “undruggable” oncogenes (e.g., Myc). Taken a step further, one could envisage using a similar approach to inform on strategies to reactivates tumor suppressor functions; for example, multiple laboratories have generated mouse models (7) in which restoration of wild-type p53 can induce tumor regression. In addition, Prembhiril and colleagues (8) have described a novel in vivo short hairpin RNA (shRNA) system capable of systematically generating such reactivation mice, using reversible knockdown of endogenous tumor suppressor genes to model robust tumor regression. These models can be used to define the “ideal states” which rational drugs such as Prima-1 can be benchmarked against to discover cotargets/therapies.

Data-Driven Approaches Are Complementary

As a prime example of an undruggable target, the exact significance of NRAS (30 years after its discovery as an oncogene) is coming into focus, but much remains enigmatic. Pathway diagrams usually involve an elaborate network of various molecules, with only the RAF–MEK–ERK and PI3K–AKT–mTOR arms generally remaining constant. Often included are pathways regulated by the small GTPases RAL, RAC, and RHO. Additional complexity is observed further downstream of the mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) pathways with respect to downstream effectors and their own complicated networks. The difficulties are compounded by the emergent recognition of differences between KRAS, NRAS, and HRAS, by cell-type and developmental stage-specific activities, and by mutations in other genes that can directly or indirectly have an impact on RAS activity. Because clinically effective pharmacologic approaches to directly target RAS have so far been unsuccessful, comprehensive analyses of RAS/MAPK signaling are required to define vulnerabilities that are shared across tumor types.

Here, we highlight three recent studies that exemplify data-driven approaches to rationally designing anti-MAPK drug combinations. First, Held and colleagues (9) conducted a large-scale combinatorial screen, testing more than 7,000 pairwise combinations of 40 drugs in 19 melanoma cell lines. Among a number of context-specific synergistic combinations, they identified simvastatin (an HMG–CoA reductase inhibitor) plus flavopiridol (a pan-CDK inhibitor) as highly efficacious in NRAS-mutant lines both in vitro and in vivo. Second, Corcoran and colleagues (10) performed an shRNA screen to identify genes that, when inhibited, could cooperate with MEKi to target KRAS-mutant cell lines. They validated BCL-2L1 knockdown and its associated inhibitor, the BH3 mimetic ABT263, as synergistic with MEKi in vivo. Finally, Duncan and colleagues (11) described a multiplatform approach to study kinome reprogramming of breast cancers in response to MEK inhibition. Multiple oncogenic kinases including AXL, and ErbB and PDGF family members were upregulated because of feedback mechanisms, and the combination of MEKi with the multikinase inhibitor sorafenib was synergistic in vivo.

From a clinical perspective, this wealth of novel potential combination therapies represents a potential boon, and the next step is to thoroughly and systematically evaluate them preclinically to bring forward into the clinic the most promising ones, linked to strong science. Indeed, the complexity and diversity of the results highlight the challenges in identifying and pursuing the most optimal strategies. In vivo data reveal that signaling downstream of pharmacologic MEK inhibition is highly complex; even with a conservative cutoff, significant changes are seen in more than 1,500 genes (4), many of which are known oncogenes or tumor suppressors. Even smaller-scale targeted assays such as phospho-receptor tyrosine kinase or pathway-specific protein arrays identify multiple potential cotargeting/combination drug targets, as described above and elsewhere (11, 12). A popular analogy is that of a game of “Whack-a-Mole,” where hitting one oncogenic target causes others to pop up and compensate (12). For example, in colorectal cancer, BRAF inhibitor monotherapy is rendered ineffective in part through compensatory activation of EGFR (13). But with hundreds of potential moles popping up after MEK inhibition, the options are either to pick the most likely targets based on prior knowledge combined with functional screening (e.g., EGFR, AKT, etc.), or to select the most statistically significant targets for screening and validation. The strength of the benchmarking approach is that the tumor regression phenotype offers the “ideal” state against which these otherwise noisy expression and posttranslational adaptive changes can be compared in an unbiased manner. This approach informs whether a given gene may be a likely cotarget or not and prioritizes the most promising strategies for preclinical validation. For example, we and others have noted that phospho-Akt is enhanced upon MEK inhibition (14). Given the known

1340 | CANCER DISCOVERY DECEMBER 2013 www.aacrjournals.org
Figure 1. Overview of the benchmark-driven analysis and the creation of cotarget databases. A, schematic comparison of a pharmacologic agent showing a partial response (Perturbation A, red) against a genetically engineered model designed to generate a complete response (Perturbation C, purple). Identifying what is missed by A allows for the identification of a cotarget drug B (blue) that, together with drug A, approximates the complete response seen in C. B, specific results of the iNRAS study (4). The large circles represent information obtained from expression and protein arrays. The RAS extinction space (purple) identified RAS-specific targets (blue) that were “missed” by the MEKi space (red). The TRAP network algorithm identified CDK4 as a cotarget regulator. The MEK plus CDK4/6 inhibitor combination thus complementarily targets the RAS extinction space. C, a generalized schematic. Oncogene extinction or tumor suppressor gene reactivation (i.e., Perturbation C, purple) generates a complete response. A pharmacologic agent (i.e., Perturbation A, red) is compared, and a key regulator(s) of pathways “missed” by A is identified via a systems biology approach. A cotarget drug B against this regulator (blue), when combined with A, would target the full space (purple). D, triplets of information are generated by the comparative analysis. Comprehensive characterization of the baseline tumor allows for correlations to be drawn between key baseline elements (e.g., mutations and epimutations) and the observed treatment responses. E, a hypothetical database of genotype-phenotype correlations, matching baseline tumor characteristics to preclinically optimized drug combinations. Such databases would inform clinical trial designs and serve as a reference for genotyped patient biopsies to rapidly predict cotargets. NRAS-mutant melanoma is depicted here as an example.
The oncogenic role of AKT, especially in RAS signaling, it is reasonable to assume that this activation might represent a rational point of coexistence. Indeed, MEK plus AKT/Pi3K inhibition has been shown to be effective preclinically (15). However, in the iNRAS model system, extinguishing NRAS genetically also results in an increase in AKT phosphorylation after 4 days, despite the full engagement of tumor regression (4). This observation suggests that AKT activation within days of MEK inhibition may not necessarily be compensatory, at least in this short-term experimental time frame. Thus, one must be cautious in interpreting the upregulation of oncogenes in response to therapy as necessarily compensatory; monitoring tumor responses provides one way to help sort the true compensatory mechanisms (i.e., those seen after pharmacologic treatment, but not in the regression state) from red herrings (i.e., those that are seen in both states, but which, by definition, do not rescue the regression state).

We note that immune therapies might also be amenable to such an approach. Data from several laboratories attest to the human relevance of GEMM immune reactions to therapy, including the influx of CD8+ T-cells seen in both a Braf<i>W161<sup>ΔN</sup>Pten<sup>-/-</sup></i> melanoma model undergoing BRAF inhibition (16) and in human patients treated with BRAF inhibitors (17). Thus, one could envision benchmarking various immune therapies (e.g., anti-CTLA4, anti–PD-1, etc.) and determining whether mechanisms of immune suppression can be overcome via cotargeting approaches. Indeed, the concept of inducible costimulator (ICOS) induction as boosting anti-CTLA4 therapy illustrates the types of synergistic pathways that might be uncovered (18). Overall, the different data-driven methods of therapy design complement one another, with the strength of the bench-marking approach lying in its ability to highlight unanticipated pathways most relevant to the complete regression phenotype.

**Signaling Activity Is Not Binary**

One other aspect of the iNRAS study supports the perspective that oncogene activity is not binary, but rather acts like a rheostat to activate various phenotypes at different thresholds of activity (4-19). Incomplete resolution of NRAS, at an intermediate time point of doxycycline withdrawal, recapitulates MEK inhibition in both the quantitative activity of extracellular signal-regulated kinase (ERK) effectors (e.g., FOSL1, MAFF, etc.) and in the phenotypic outputs of activated apoptosis but not cell-cycle arrest. In other words, one reason that MEKis are inefficient in the setting of apoptosis but not cell-cycle arrest. In other words, one reason that MEKis are inefficient in the setting of tumor regression state (4). This observation suggests that AKT activation within days of MEK inhibition may not necessarily be compensatory, at least in this short-term experimental time frame. Thus, one must be cautious in interpreting the upregulation of oncogenes in response to therapy as necessarily compensatory; monitoring tumor responses provides one way to help sort the true compensatory mechanisms (i.e., those seen after pharmacologic treatment, but not in the regression state) from red herrings (i.e., those that are seen in both states, but which, by definition, do not rescue the regression state).

We note that immune therapies might also be amenable to such an approach. Data from several laboratories attest to the human relevance of GEMM immune reactions to therapy, including the influx of CD8+ T-cells seen in both a Braf<i>W161<sup>ΔN</sup>Pten<sup>-/-</sup></i> melanoma model undergoing BRAF inhibition (16) and in human patients treated with BRAF inhibitors (17). Thus, one could envision benchmarking various immune therapies (e.g., anti-CTLA4, anti–PD-1, etc.) and determining whether mechanisms of immune suppression can be overcome via cotargeting approaches. Indeed, the concept of inducible costimulator (ICOS) induction as boosting anti-CTLA4 therapy illustrates the types of synergistic pathways that might be uncovered (18). Overall, the different data-driven methods of therapy design complement one another, with the strength of the benchmarking approach lying in its ability to highlight unanticipated pathways most relevant to the complete regression phenotype.

**Signaling Activity Is Not Binary**

One other aspect of the iNRAS study supports the perspective that oncogene activity is not binary, but rather acts like a rheostat to activate various phenotypes at different thresholds of activity (4-19). Incomplete resolution of NRAS, at an intermediate time point of doxycycline withdrawal, recapitulates MEK inhibition in both the quantitative activity of extracellular signal-regulated kinase (ERK) effectors (e.g., FOSL1, MAFF, etc.) and in the phenotypic outputs of activated apoptosis but not cell-cycle arrest. In other words, one reason that MEKis are inefficient in the setting of tumor regression state (4). This observation suggests that AKT activation within days of MEK inhibition may not necessarily be compensatory, at least in this short-term experimental time frame. Thus, one must be cautious in interpreting the upregulation of oncogenes in response to therapy as necessarily compensatory; monitoring tumor responses provides one way to help sort the true compensatory mechanisms (i.e., those seen after pharmacologic treatment, but not in the regression state) from red herrings (i.e., those that are seen in both states, but which, by definition, do not rescue the regression state).

We note that immune therapies might also be amenable to such an approach. Data from several laboratories attest to the human relevance of GEMM immune reactions to therapy, including the influx of CD8+ T-cells seen in both a Braf<i>W161<sup>ΔN</sup>Pten<sup>-/-</sup></i> melanoma model undergoing BRAF inhibition (16) and in human patients treated with BRAF inhibitors (17). Thus, one could envision benchmarking various immune therapies (e.g., anti-CTLA4, anti–PD-1, etc.) and determining whether mechanisms of immune suppression can be overcome via cotargeting approaches. Indeed, the concept of inducible costimulator (ICOS) induction as boosting anti-CTLA4 therapy illustrates the types of synergistic pathways that might be uncovered (18). Overall, the different data-driven methods of therapy design complement one another, with the strength of the benchmarking approach lying in its ability to highlight unanticipated pathways most relevant to the complete regression phenotype.

**Signaling Activity Is Not Binary**

One other aspect of the iNRAS study supports the perspective that oncogene activity is not binary, but rather acts like a rheostat to activate various phenotypes at different thresholds of activity (4-19). Incomplete resolution of NRAS, at an intermediate time point of doxycycline withdrawal, recapitulates MEK inhibition in both the quantitative activity of extracellular signal-regulated kinase (ERK) effectors (e.g., FOSL1, MAFF, etc.) and in the phenotypic outputs of activated apoptosis but not cell-cycle arrest. In other words, one reason that MEKis are inefficient in the setting of tumor regression state (4). This observation suggests that AKT activation within days of MEK inhibition may not necessarily be compensatory, at least in this short-term experimental time frame. Thus, one must be cautious in interpreting the upregulation of oncogenes in response to therapy as necessarily compensatory; monitoring tumor responses provides one way to help sort the true compensatory mechanisms (i.e., those seen after pharmacologic treatment, but not in the regression state) from red herrings (i.e., those that are seen in both states, but which, by definition, do not rescue the regression state).
oncogene extinction or tumor suppressor reactivation, inducible shRNA or expression plasmids could be used to define the molecular state corresponding to a desired efficacy, such as tumor regression. Ultimately, correlations between the known baseline genotypes of tumors and their optimal drug combination will allow us to generate a database of therapies tailored to specific mutational profiles both within and across tumor types (Fig. 1E).

A second aspect to bridging toward the clinic is in anticipating how to consistently model complete regression in the laboratory. For example, not all KRAS-mutant cancers rely on KRAS: when KRAS expression is extinguished by potent shRNAs in human lung and pancreatic cell lines, nearly half of the lines tested were found to be KRAS-independent, as shRNA knockdown failed to induce apoptosis (22), reflecting a differential dependence on mutant KRAS signaling to maintain growth. This spectrum of responses may be generally true for most, if not all, oncogenes and tumor suppressors. In such cases, it will be necessary to identify and overcome the underlying independence-conferring mechanism(s) to properly generate complete regression phenotypes. In the above-referenced KRAS study, epithelial–mesenchymal transition (EMT) was identified as a pervasive signature throughout the KRAS-independent cell lines (22). Indeed, many recent publications have highlighted a similar correlation between EMT status and a wide range of drug resistances. However, it is likely that this represents only one of many mechanisms of oncogene independence. Overall, resensitizing agents would be necessary in oncogene-independent samples for generating the tumor regression state, additionally acting as a cotargeting agent.

In summary, accounting for mutational heterogeneity and consistent benchmarking will require a large-scale investment that a consortial effort can create, with the capacity to potentially provide a clinic-ready, biologically significant knowledge base of tumor genotypes and their matched, optimized drug combination therapies.

**Personalizing Therapeutic Combinations in the Clinic**

The coclinical trial concept represents an attractive transition from laboratory to clinic (23). In such a trial, biopsies of a patient’s tumor are passed through preclinical model systems (e.g., mouse), and the response of the tumor to the same targeted therapy being administered to the patient is monitored. From there, genomic analyses—sequencing and expression profiling—can make several important determinations. First, expansion and drug dosing of patient-derived xenografts will provide models that can be quickly cross-referenced to the preclinical knowledge base to predict rational cotargeting strategies. Second, such a coclinical trial can provide early predictions of response by allowing for a rapid molecular assessment of response and/or biologic markers. Third, the expression assessment can determine whether the patient xenograft is behaving as predicted by the preclinical knowledge base, or whether novel combinations need to be quickly uncovered. In this last regard, it should also be possible to establish patient-derived cell cultures *ex vivo*, transfected with the appropriate vector (oncogene knockdown or tumor suppressor expression), and perform xenografting to establish a truly personalized drug combination by benchmarking. Ultimately, the coclinical information will be routed back to engage the optimal course of treatment for the patients.

The ultimate clinical application of this approach to designing personalized combination therapy is to be able to monitor, noninvasively and in real time, the response of a patient and his/her tumor to a targeted pharmaceutical compared with a benchmark model. Guided by the preceding preclinical and coclinical data, physicians would be able to make informed decisions about efficacious, rational drug combinations in both predictive and reactive manners. The ability to longitudinally monitor important phenotypes (such as various hallmarks of cancer in a tumor) in response to a treatment would provide the rational basis to customize additional drug(s) in combination for each individual patient. For example, if proliferation and apoptosis could be monitored in a clinically relevant time frame, one can imagine treating a NRAS-mutant melanoma patient first with a MEKi, followed by evidence-based selection of a CDK4 inhibitor as a combination, based on the observation that proliferation was not inhibited. Alternatively, it is also possible that, in a separate patient with a different genomic makeup of a NRAS-mutant melanoma, MEKi is able to shut off proliferation but apoptosis is not activated; in such a patient, the personalized combination could be an AKT or BCL2 inhibitor. The capability to longitudinally monitor a patient’s response as envisioned means a data-driven approach to customized combinations can address both the passive and adaptive mechanisms of drug resistance to initial therapies in a timely manner. Although it is not yet available today, we believe such real-time clinical monitoring is on the horizon, given the rapidly advancing technologies in imaging and noninvasive biomarker discovery.

**CONCLUSIONS**

The advent of targeted therapies has allowed for the tailoring of clinical care based on driver genetic lesions in a patient’s DNA; for example, vemurafenib in melanoma targets mutant BRAF, imatinib mesylate in various cancers targets ABL, or PDGFR translocations or KIT amplifications. However, the full promise of such targeted therapy has not yet been realized in part due to the powerful adaptive responses of the tumors. The benchmark-driven paradigm to define cotargeting strategies has the potential to design optimized combinations that are customized to individual patients. Leveraging model systems to establish benchmarks for tumor regression or other desired states in a preclinical or coclinical trial context offers a first step toward translating such a paradigm—and associated *in vivo* systems biology approaches—to clinical application. This will require a coordinated and systematic effort among preclinical modelers and biologists, genomic scientists, and computational modelers, similar to those represented by the National Cancer Institute’s (NCI) MMHCC (Mouse Model of Human Cancer Consortium), TCGA (The Cancer Genome Atlas), and ICBP (Integrative Cancer Biology Program). Furthermore, such an effort will require a new model of cooperation and collaboration between academia and industry to ensure that these studies are conducted to industry standards and that the results are clinically
actionable and available as a public resource. Finally, this must be accompanied by educational efforts so that our clinical colleagues can optimize their care of patients and, importantly, feedback to teach and improve these exploratory studies conducted by the research communities.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The authors thank Ron DePinho and Carlo Toniatti for critical readings of the article.

Grant Support

This review was supported in part by an American Cancer Society Postdoctoral Fellowship (117842-PF-09-261-01-TBG) to L.N. Kwong, and a Mouse Models of Human Cancer Consortium UO1 grant (CA141308-05), an NIH ROI grant (CA93947-10), and a Texas Prevention & Research Institute of Texas grant (R1204).

Published online December 10, 2013.

REFERENCES

3. Schnellmann RG, Stommel JW, Xu L, Zhang X, et al. & a Mouse Models of Human Cancer Consortium UO1 grant (CA141308-05), an NIH ROI grant (CA93947-10), and a Texas Prevention & Research Institute of Texas grant (R1204).
11. Duncan JS, Whittle MC, Nakamura K, Abell AN, Midland AA, Zawistowski JS, et al. Dynamic reprogramming of the ki67/nuclear expression and a Mouse Models of Human Cancer Consortium UO1 grant (CA141308-05), an NIH ROI grant (CA93947-10), and a Texas Prevention & Research Institute of Texas grant (R1204).
A Systems Biology Approach to Personalizing Therapeutic Combinations

Lawrence N. Kwong, Timothy P. Heffernan and Lynda Chin


Access the most recent version of this article at:
http://cancerdiscovery.aacrjournals.org/content/3/12/1339

This article cites 23 articles, 9 of which you can access for free at:
http://cancerdiscovery.aacrjournals.org/content/3/12/1339.full#ref-list-1

Sign up to receive free email-alerts related to this article or journal.

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.